

Changes in carotenoid intake in the United States: The 1987 and 1992 National Health Interview Surveys

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ABSTRACT

Objectives To assess the changes in carotenoid intake between 1987 and 1992 among US adults by sociodemographic characteristics and high-risk groups for chronic disease; and to identify the dietary sources of specific carotenoid intake.

Design A food frequency questionnaire (FFQ) was collected from a representative sample of respondents to the 1987 and 1992 National Health Interview Surveys throughout two calendar quarters. Black and white adults, 18 to 69 years old, participated in 1987 (n=8,161) and 1992 (n=8,341).

Method FFQ data were matched and linked to the US Department of Agriculture-National Cancer Institute carotenoid food composition database for analysis.

Statistical analysis Mean differences in carotenoid intake over time were compared by sociodemographic characteristics and region of the country, after adjustment for sampling weights in a multiple linear regression model.

Results Mean intake of the carotenoid lutein declined among white women (18%), among adults aged 40 to 69 years (16%), among persons with 9 to 12 years of education (11%), among nondrinkers (18%), among drinkers of 1 to 6 alcoholic drinks/week (7%), among smokers (former smokers by 11%, current smokers by 7%, and never smokers by 9%), among income groups (<\$20,000 by 7%, ≥\$20,000 by 9%), and residents in the south and northeast (by 13% each, respectively). Mean intake of the carotenoid lycopene increased among white men (9%), among adults aged 18 to 39 years and aged 40 to 69 years (by 5% and 6%, respectively), among those with 13 years of education or more (12.5%), among alcohol drinkers (by 10% and 7% for 1 to 6 vs 7 or more drinks/week, respectively), among former and current smokers (by 6% each), among those with incomes ≥\$20,000 (8%), and among residents in the west (16%) and midwest (5%). All differences described were statistically significant ($P < .01$).

Application The decline in lutein intake (from dark green leafy vegetables), particularly in white women, may have public health implications as a result of the recognized inverse association between carotenoid intake and disease risk. *J Am Diet Assoc.* 1997;97:991-996.

Epidemiologic research has demonstrated a protective effect of high fruit and vegetable consumption on the risk of chronic diseases (1-3). Consumption of foods such as tomatoes, carrots, pumpkin, spinach, dark green leafy vegetables, green peppers, and broccoli has been inversely related to the risk of cancers after adjustment for risk factors such as alcohol or tobacco (4). Their protective effect has been attributed to the antioxidative capacity of carotenoids and other phytochemical components, which are highly concentrated in these foods (5).

Carotenoids exhibit preventive capacities in the body and play an essential role in normal epithelial cell differentiation and maintenance (5). Provitamin A carotenoids such as beta carotene are precursors of retinol and retinoids (5). Carotenoids (eg, beta carotene, lycopene) are able to quench the free radicals produced by normal aerobic metabolism, which are capable of damaging DNA, proteins, lipids, and carbohydrates (6,7).

Fruits and vegetables are the primary source of carotenoids in the US diet. Beta carotene is available in foods such as cantaloupe, carrots, sweet potatoes, dark green leafy vegetables, green salad, broccoli, and vegetable soup. The highest concentration of lutein is found in dark green leafy vegetables (eg, kale, collards, mustard greens), spinach, dark green lettuce, and broccoli. Lycopene is responsible for the red color in fruits such as tomato or pink grapefruit (8).

Baseline survey data from the 5 A Day for Better Health Program indicated that only 23% of US adults were consuming the minimum recommended five servings of fruits and vegetables per day (9). Median fruit and vegetable consumption stands at 3.5 servings per day for most adults (9). However, segments of the adult population (eg, current smokers, those with less education, or those with lower incomes) are short of meeting the minimum fruit and vegetable intake recommendations by at least two servings a day (9).

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Efforts to improve fruit and vegetable consumption in the United States began with the California 5 A Day Program in 1988. This unique program combined resources from state and local health departments, public health groups, the produce industry, and retail grocery markets to promote the message to eat five or more servings of fruits and vegetables a day (10). This program was expanded to a national campaign in 1991 with the support of the National Cancer Institute (NCI) and the Produce for Better Health Foundation (10).

For health promotion campaigns to be effective, an appropriate message should be defined and targeted toward the intended audience (11,12). Whether the 5 A Day message to promote the benefits of increased fruit and vegetable consumption has reached its intended audience is still under investigation (13,14). Using data from the 1987 and the 1992 National Health Interview Survey (NHIS) (15) that were coincident with the initiation of the 5 A Day Program, our objectives here were to describe changes in specific carotenoid intakes from 1987 to 1992 by sociodemographic characteristics and groups at high risk for chronic disease; and to identify the fruit and vegetable sources related to the changes in carotenoid intakes.

METHODS

National Health Interview Surveys

The 1987 NHIS and the 1992 NHIS, which included the NCI-sponsored Cancer Epidemiology Study Supplement were designed as stratified multistage cluster samples representative of households within the 50 states (15,16). Only households with resident, noninstitutionalized US civilians were sampled. Experienced Census Bureau staff administered the survey questionnaire to adults aged 18 to 99 years in home interviews. One adult was randomly selected from each household to answer the questionnaire, which included basic health, demographic, and dietary information. Telephone interviews were conducted after unsuccessful repeated efforts to reach respondents at home (15). The response rates of the eligible households in the sample were 82% in 1987 ($n=22,080$) and 86% in 1992 ($n=12,005$) (15,16).

Data analysis focused on blacks and whites. Hispanics and other minorities were excluded from the analysis because of small sample sizes and the omission of ethnic-specific foods from the food frequency questionnaire (FFQ). Because the second National Health and Nutrition Examination Survey (NHANES II) (17) population (18 to 69 years old) was used in weighting the carotenoid intakes, our sample for this data analysis was limited to the same age distribution. Finally, budgetary constraints in 1992 stopped data collection of the Cancer Epidemiology Study Supplement at the end of quarter 2. We conducted a multiple linear regression analysis of the 1987 data and found that beta carotene and lycopene intakes were significantly higher in the first two quarters than in the last two quarters (data not shown). Therefore, data analyses were restricted to the responses from blacks and whites aged 18 to 69 years during the first two calendar quarters of each year (1987: $n=8,161$ and 1992: $n=8,341$).

Dietary Analysis

Dietary data were collected using a 60-item FFQ in 1987 and a 74-item FFQ in 1992. These questionnaires were shortened versions of the Block semiquantitative FFQ (18). Subjects reported their usual frequency of intake and portion size of specified items during the past year (18). Because an FFQ provides a relative measure of intake, not a measure of a person's absolute intake, the reported estimated mean carotenoid intakes were used for comparison by subgroups.

Estimation of Dietary Carotenoid Intake

The fruit and vegetable items in the 1992 FFQ were matched to the same items in the 1987 FFQ for comparability. All carotenoid-rich food items were linked to the US Department of Agriculture-NCI (USDA-NCI) food composition database to determine carotenoid content (alpha carotene, beta carotene, beta cryptoxanthin, lutein plus zeaxanthin, and lycopene) (8,19,20). For all carotenoid-rich food items in the FFQ, each set of foods contained within the questionnaire item (eg, broccoli: fresh, frozen, cooked) was matched to a similar group of foods from the 24-hour recalls in NHANES II collected between 1976 and 1980, (which represents a time lag between the NHANES II and NHIS data) (21). The frequency of intake of these grouped foods as reported in NHANES II was calculated and weighted to reflect the total US population by age and gender. The specific carotenoid value for each food in the NHIS questionnaire item was then multiplied by this weighted frequency, which reflects for example the relative proportions of fresh and frozen broccoli, from NHANES II. Next, the respondents' reported frequency of intake during the past year and portion size for each NHIS questionnaire item was multiplied by the weighted individual carotenoid values for each set of foods. Finally, the sum of the respondent's item-specific carotenoid intakes throughout the year were averaged to provide an estimated mean daily dietary intake (milligrams per day) (20).

Data and Statistical Analysis

The statistical analyses involved several steps. First, differences in demographic and lifestyle characteristics between the populations sampled in 1987 and 1992 were tested for statistical significance using χ^2 tests. Continuous variables were grouped into the following categories: age (18 to 39, 40 to 69 years), education (0 to 8, 9 to 12, 13 or more years), alcohol consumption (0, 1 to 6, 7 or more drinks per week), cigarette smoking (never, former, current), and income ($< \$20,000$, $\geq \$20,000$). The categorical variables of race, gender, and region of the country (west, midwest, south, northeast) were also included. Data responses coded as missing or unknown were excluded from the χ^2 analysis. The variable categories were selected so as to determine if variation existed within recognized confounders of dietary intake of fruits and vegetables (18,20).

Second, the mean carotenoid intake and its standard error for each demographic and lifestyle covariate were compared over time using t tests to determine significant differences. Finally, a multiple linear regression model, which included all the covariates as independent variables, tested for interactions between years and all covariates. All findings were considered statistically significant with a two-sided P value $< .01$ to help protect against inflated type I errors due to multiple comparisons.

All analyses were computed with observations weighted by their sample weights and with standard errors estimated by taking account of the stratification, clustering, and weighting of the sample selection. The software package SUDAAN (version 6.4, 1995, Research Triangle Institute, Research Triangle Park, NC) was used in the analyses.

RESULTS

The 1987 and 1992 analytic subpopulations had similar distributions by race, gender, smoking, and region of the country (Table 1). However, the 1992 population was slightly older, had more education, drank less frequently, and had a higher level of income than the 1987 population ($P < .001$).

The results focus on beta carotene, lutein, and lycopene intakes because these carotenoids form the highest percentage of total carotenoid intake in the diet and are the primary

Table 1

The weighted percent (sample size)* differences in demographic and lifestyle characteristics between the 1987 and 1992 subpopulations for calendar quarters 1 and 2 as tested using χ^2 test for independence

Characteristic	1987 quarter 1 and 2		1992 quarter 1 and 2	
	%	No.	%	No.
Race^b				
Black	11	1,190	12	1,222
White	89	6,971	88	7,119
Age* (y)				
40-69	46	3,681	48	3,906
18-39	54	4,480	52	4,435
Gender^b				
Women	52	4,657	52	4,659
Men	48	3,504	48	3,682
Education** (y)				
0-8	7	484	6	377
9-12	67	4,193	65	3,960
13+	26	1,703	29	1,854
Alcohol** (drinks/wk)				
7 or more	20	1,670	18	1,481
1-6	54	4,378	45	3,654
None	26	2,113	37	2,994
Smoking^b				
Former	22	1,722	23	1,826
Current	32	2,608	30	2,448
Never	46	3,635	47	3,855
Income** (\$)				
≥20,000	68	4,866	75	5,395
<20,000	32	3,161	25	2,590
Region of country^b				
West	18	1,529	18	1,554
Midwest	26	2,182	28	2,240
South	34	2,781	33	2,684
Northeast	22	1,669	21	1,651

*Complete data set: n=8,161 in 1987 and n=8,341 in 1992. The variation in total numbers was caused by missing observations (ie, respondent answered question as unknown), which were excluded within each category for data analysis.

^bNot significant.

*P=.001.

**P=.0001.

components of total circulating plasma carotenoid levels. Between 1987 and 1992, blacks and whites experienced a similar pattern of change in carotenoid intakes, but the decline in lutein intake (by 10%) and increase in lycopene intake (by 6%) were only significant for whites (Table 2). Whereas beta carotene (15%) and lycopene (9%) intakes increased among men, beta carotene (8%) and lutein intake (16%) declined among women.

In adults aged 40 to 69 years, lutein intake declined (by 16%) and lycopene intake increased (by 6%) over time. Lycopene intake increased (by 5%) in younger adults aged 18 to 39 as well. Lutein intake declined (by 11%) among those with 9 to 12 years of education and lycopene intake declined by a similar percentage among those with 0 to 8 years of education. However, lycopene intake increased (by 13%) among those with 13 years of education or more (Table 2).

Among alcohol groups, persons who never drank decreased their intakes of beta carotene (by 6%) and lutein (by 18%) (Table 2). Those who drank 1 to 6 drinks/week increased their beta carotene intake by 5%, increased their lycopene intake by 10%, and decreased their lutein intake by 7%. Those who consumed 7 or more drinks/week experienced a 6% increase in

beta carotene intake and a 7% increase in lycopene intake. When the population was categorized by smoking status or by income category, lutein intake significantly declined across all strata. In contrast, lycopene intake increased among former and current smokers (by 6%) as well as among those with an income ≥\$20,000 (by 8%).

Changes in carotenoid intakes by region of the country indicated an increase in beta carotene intake in the west (by 7%) and midwest (by 13%); an increase in lycopene intake in the west (16%) and midwest (5%); and a decline in lutein intake (by 13%) in the south and northeast (Table 2).

After stratifying by race and gender, carotenoid intakes among whites, but not blacks, varied by gender. White men significantly increased beta carotene intake (by 16%) and lycopene intake (by 9%) (Table 2). White women decreased consumption of beta carotene (by 9%) and lutein (by 18%). Between 1987 and 1992, white women reduced their consumption of lutein-rich vegetables (eg, spinach, green salads, and green cabbage) and beta carotene-rich fruits (eg, cantaloupe, citrus fruits), whereas white men increased their consumption beta carotene-rich foods (eg, carrots, broccoli) and lycopene-

Table 2

Mean±(standard error) differences in carotenoid (beta carotene, lutein, lycopene) intake plus percent change in intake between 1987 (n=8,161) and 1992 (n=8,341) National Health Interview Survey data by sociodemographic characteristics, region of the country, and stratification within race and gender^a

	1987 Beta carotene	1992 Beta carotene	% Change	1987 Lutein	1992 Lutein	% Change	1987 Lycopene	1992 Lycopene	% Change
	mg/day			mg/day			mg/day		
Race									
White	2.56±0.03	2.63±0.03	2.6	1.00±0.03	1.79±0.26	-9.7***	2.09±0.02	2.22±0.02	6.0***
Black	3.22±0.11	3.24±0.09	0.6	3.69±0.13	3.41±0.13	-7.7	1.94±0.06	2.01±0.07	3.5
Gender									
Men	2.56±0.04	2.93±0.05	15.0***	2.15±0.05	2.15±0.05	0.4	2.10±0.03	2.29±0.03	9.0***
Women	2.71±0.04	2.48±0.03	-8.4***	2.21±0.04	1.86±0.03	-16.0***	2.06±0.03	2.10±0.03	2.1
Age (y)									
18-39	2.34±0.03	2.42±0.04	3.4	1.95±0.04	1.95±0.04	-0.4	2.07±0.03	2.18±0.03	5.3**
40-69	2.99±0.05	3.01±0.04	0.7	2.46±0.05	2.06±0.04	-16.4***	2.09±0.03	2.22±0.03	5.8**
Education (y)									
0-8	2.91±0.13	2.64±0.13	-7.6	2.31±0.12	2.06±0.20	-9.2	2.07±0.09	1.83±0.09	-11.0*
9-12	2.56±0.10	2.50±0.08	0.2	2.29±0.11	1.92±0.07	-11.0***	1.97±0.05	2.00±0.06	3.5
13 or more	2.50±0.05	2.52±0.05	6.4	2.00±0.04	1.78±0.04	-6.2	2.00±0.03	2.00±0.04	12.5***
Alcohol (drinks/week)									
Never	2.82±0.05	2.65±0.06	-5.7*	2.29±0.05	1.88±0.04	-18.0***	2.05±0.04	2.03±0.04	-0.6
1-6	2.56±0.04	2.70±0.04	5.4*	2.11±0.03	1.97±0.04	-6.7**	2.05±0.03	2.25±0.03	10.0***
7 or more	2.62±0.08	2.79±0.06	6.4*	2.26±0.08	2.30±0.08	1.9	2.20±0.04	2.36±0.05	7.0*
Smoking									
Never	2.67±0.04	2.71±0.05	1.3	2.18±0.04	1.98±0.05	-9.0**	2.12±0.03	2.20±0.03	4.0
Former	2.88±0.07	3.02±0.06	5.1	2.37±0.08	2.10±0.05	-11.0**	2.17±0.05	2.31±0.05	6.4*
Current	2.43±0.05	2.44±0.05	0.5	2.07±0.05	1.92±0.06	-7.4*	1.95±0.03	2.08±0.04	6.3**
Income (\$)									
<20,000	2.64±0.05	2.64±0.06	-0.3	2.23±0.05	2.08±0.06	-7.0*	2.10±0.03	2.08±0.04	-1.2
≥20,000	2.65±0.04	2.73±0.04	3.1	2.16±0.04	1.96±0.03	-9.0***	2.07±0.03	2.24±0.02	8.3***
Region of country									
West	2.79±0.06	2.97±0.08	6.5*	2.00±0.08	2.09±0.07	4.7	1.98±0.04	2.31±0.04	16.4***
Midwest	2.17±0.06	2.25±0.05	13.0***	1.72±0.05	1.65±0.06	-3.6	1.91±0.04	2.01±0.03	5.3*
South	2.74±0.06	2.70±0.06	-1.2	2.54±0.06	2.21±0.07	-13.0***	2.01±0.03	2.09±0.04	4.2
Northeast	2.92±0.05	2.82±0.05	-3.4	2.32±0.07	2.02±0.05	-12.8**	2.46±0.05	2.49±0.06	1.3
White men	2.46±0.05	2.84±0.05	16.0***	1.96±0.05	1.95±0.04	-0.5	2.11±0.03	2.31±0.03	9.3***
White women	2.67±0.04	2.42±0.04	-9.1***	2.02±0.03	1.65±0.03	-18.3***	2.08±0.03	2.14±0.03	2.6
Black men	3.39±0.24	3.64±0.17	7.5	3.79±0.25	3.64±0.20	-4.2	2.02±0.11	2.18±0.12	7.7
Black women	3.08±0.12	2.91±0.11	-5.7	3.60±0.14	3.21±0.13	-10.8	1.88±0.68	1.87±0.79	-0.3

^aData (1976-1980) from the second National Health and Nutrition Examination Survey (17) were used for weighting the 1987 and the 1992 National Health Interview Survey dietary intakes.

*P<.01.

**P<.001.

***P<.0001.

en 1987
nd strat-

Change

6.0***
3.5

9.0***
2.1

5.3**
5.8**

11.0*
3.5
12.5***

-0.6
10.0***
7.0*

4.0
6.4*
6.3**

-1.2
8.3***

16.4***
5.3*
4.2
1.3

9.3***

2.6

7.7

-0.3

Health In-

rich foods (eg, tomato, tomato-based pasta sauce, pizza sauce, pink grapefruit). Overall, black women and men reported consuming a minimum of one serving per week more of dark green leafy vegetables and one-half serving per week more of sweet potato than white women and men (data not shown).

To test for statistically significant differences in patterns of changes between 2 years by specific variables, a model for interactions was used to examine the changes in carotenoid intake and each covariate after adjustment for all others (data not shown). Significant interactions appeared between years and the following categories: gender, age, and region of the country. Among the gender-specific interactions, the increase in beta carotene and lutein intake in men between 1987 and 1992 was significantly different from the decrease seen in women ($P<.0001$). Age-specific interactions with lutein intake over time revealed that the decline in the 40- to 69-year-old group was significantly greater than the decline in the 18- to 39-year-old group ($P<.0001$). Between 1987 and 1992, the increase in beta carotene intake in the west was significantly different from the decline in the south ($P<.01$), whereas the increase in the midwest was different from both the decline in south ($P<.001$), and in the northeast ($P<.001$). Similar to beta carotene, the increase in lutein intake over time in the west was significantly different from the decline noted in the south ($P<.001$) and the northeast ($P<.001$). Interactions of lycopene intake over time by region of the country indicated that the increase in the west was significantly greater than the increases in the northeast ($P<.001$), the south ($P<.001$), and the midwest ($P<.0001$).

DISCUSSION

Between 1987 and 1992, the US population experienced changes in specific carotenoid intake by race, gender, and other lifestyle characteristics. Whereas blacks and whites demonstrated a decrease in lutein intake and an increase in lycopene intake, these changes were only statistically significant for whites. Lutein intake declined among white women, among the older age group, among those with 9 to 12 years of education, among nondrinkers and low-to-moderate drinkers (1 to 6 drinks/week), among all income groups, and among residents of the south and northeast. Moreover, lutein intake declined across smoking groups, even in the presence of a decline in smoking in the sample population.

Lycopene intake increased among white men, among all age groups, among those with 13 or more years of education, among alcohol drinkers (1 to 6 and 7 or more drinks/week), among former and current smokers, among those with an income $\geq \$20,000$, and among residents of the west and midwest. The changes in carotenoid intakes between 1987 and 1992 by gender, age, and region of the country remained significant after accounting for interaction effects by year.

Region-specific differences reflected increases in beta carotene, lutein, and lycopene in the west and either a decline in or significantly lower increase in other regions. The fact that residents in the west have more clearly "heard" the message to increase intake of carotenoid-rich foods might be an indication that the 5 A Day Program, which began in California, has had time to distribute the message across the region. Future national surveys like the NHIS need to include questions about 5 A Day and other regional and national programs to determine whether the message is being translated into nutritional change.

The decline in beta carotene and lutein intakes for white women from 1987 to 1992 may reflect earlier reported variations in carotenoid-rich fruit and vegetable intake. Based on a comparison of data from the 1977-78 Nationwide Food Consumption Survey (22) and the 1985 Continuing Survey of Food

Intake by Individuals (CSFII) (23), US women aged 19 to 50 years have diversified their diet over time, but also decreased consumption of certain foods (by 5 to 10 g per person per day) including: high-fat meats, eggs, higher-fiber vegetables (eg, corn, green beans, peas, lima beans, cooked cabbage), and green and yellow vegetables (eg, broccoli, spinach, other mixed greens, carrots, winter squash) (24). On the basis of data from the 1989-1991 CSFII (25), more than 50% of all adults reported that they had consumed less than one serving of fruit and 8% of adults had less than a single serving of vegetables per day. Women were more likely than men to have had less than a single serving of vegetables per day (26). Because vegetables are rich sources of carotenoids, flavonoids, fiber, and other phytochemicals, a reduction in their consumption could lead to an increased risk of disease.

Our results indicate that lutein intake declined between 1987 and 1992; however, consumption of lycopene-rich foods and beta carotene-rich foods has increased

Discrepancies between published reports may be attributable to differences in population sample size and characteristics, dietary methodologies, and classifications of fruits and vegetables (10,24,26-28). Higher mean intakes of fruits and vegetables were reported when a broader classification of vegetables (eg, including french fries, which contribute approximately 0.4 servings/day, and legumes/beans) was applied to the 1989-1991 CSFII (28). A mean intake of 4.3 servings of fruits and vegetables per day was reported in 32% of 8,181 US adults in the 1989-1991 CSFII. However, compliance with recommended guidelines for 5 servings per day declined (to 24% for fruits and 12% for vegetables) in adults when more stringent standards of vegetable and fruit classifications were used in the same population (28).

The use of NHANES II data for weighting the food sources constitutes a time lag in data collection between NHIS and NHANES II. During the time of these analyses, NHANES II was the most comprehensive database available for comparison with the 1987 and 1992 NHIS; thus, it was chosen for weighting purposes. USDA food supply data indicate that the annual per capita consumption of fruits and vegetables did increase up to 20% as a percentage of total fruit and vegetable use over that time (29). This variation in the proportion of foods in each food group selected to weight the NHIS data is not anticipated to notably influence the comparison of carotenoid consumption patterns at a population level because the difference in carotenoid content between the selected groups of foods (eg, fresh and frozen broccoli) is minimal. Ideally, a detailed assessment of more mixed-food dishes, such as pizza, would have been desirable. Unfortunately, the 1987 NHIS FFQ did not query specifically for such items, making detailed comparisons over time impossible for certain mixed-item dishes.

Additionally, research based on data from an FFQ has limitations because of the poor sensitivity to cultural differences in diet and comprehensive assessment of all fruit and vegetable intakes. The use of 24-hour records or recalls may be able to detect possible cultural differences or specify portion size in more detail than an FFQ (30). Unfortunately, the costs of conducting and analyzing 24-hour records may prohibit their use in large-scale surveys. Despite potential limitations to the FFQ, the fruit and vegetable intake patterns reported here are similar to results from investigators using more detailed dietary tools (10,26,27).

APPLICATION

Our results indicate that lutein intake declined between 1987 and 1992, particularly among white women. However the consumption of lycopene-rich foods (eg, tomato, tomato-based pasta sauce, pizza sauce, and pink grapefruit) and beta carotene-rich foods has increased, particularly in the western region of the United States. Moreover, blacks consume, on average, one or more servings of carotenoid-rich fruits and vegetables per week more than whites. These findings are in accord with the results of others (21,24) and identify a new population subgroup for nutrition education—white women. For the purposes of nutrition education of the population, it is important to know what potential factors may be influencing food choices within this subgroup. Better knowledge of eating practices will allow health educators and nutritionists to target survey questions as well as nutrition information more effectively to this subgroup to reduce their risk for chronic disease. ■

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